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OF THE

INDIAN ACADEMY

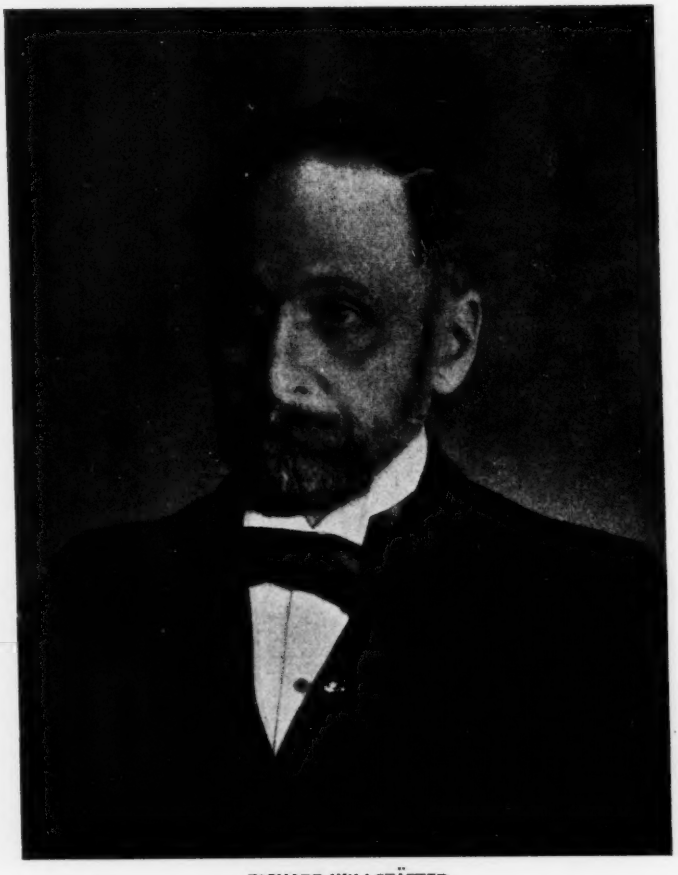
OF SCIENCES

VOL. XVII] *SECTION A* [No. 5

MAY, 1943

Price Rs. 2 or 3 Sh. *Annual Subscription Rs. 18*

PRINTED AT THE BANGALORE PRESS, BANGALORE CITY, BY G. SRINIVASA RAO, SUPERINTENDENT, AND PUBLISHED BY THE INDIAN ACADEMY OF SCIENCES, BANGALORE.



RICHARD WILLSTÄTTER

RICHARD WILLSTÄTTER

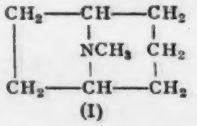
By the death of R. Willstätter on the 3rd of August 1942 the world has lost one of the greatest masters of organic chemistry and one of the most distinguished members of the famous Munich school originated by Adolph von Baeyer. Willstätter was born on the 13th of August 1872 in Carlsruhe in Germany. His chemical education was in the University of Munich, the greatest classical centre of chemistry in Europe. The early inspiration that he received from von Baeyer and his school is reflected all through his epoch-making investigations in after-life. He took his Doctor's degree in Philosophy in 1894 and continued to work as Privatdozent in Munich till 1902. Subsequently he was appointed as Professor at the Polytechnic in Zurich. From 1912 to 1915, he held the directorship of the Kaiser Wilhelm Institute for chemistry in Berlin and an honorary professorship at the Berlin University. In 1915, he accepted the invitation to return to his own *alma mater* in Munich and occupy the chair vacated by his renowned professor, von Baeyer. After a few years he retired from this professorship and continued his career as a chemical investigator in his private laboratories in Munich itself.

Within the limits set for this obituary notice, it is not possible to do justice to the great work of such an eminent personality as Willstätter. An attempt is therefore made to touch upon only the greatest of his achievements—in a way to have a review of the great peaks ignoring the comparatively minor heights. His main work lay in that part of organic chemistry which is intimately connected with biology, and along with Emil Fischer he was largely responsible for the rapid development of biochemistry as a new branch of chemistry. Roughly, his sojourn in different centres of chemistry is associated with his great work in different fields. His earlier work on alkaloids was done in Munich; Zurich is associated with his study of chlorophyll; in Berlin the anthocyanins were examined and the later Munich period was concerned mainly with enzymes.

Tropane Group of Alkaloids

The earliest work of fundamental importance undertaken by Willstätter related to the tropane group of alkaloids. It was done mainly during the years 1894–1903, though he later reverted to it to some extent even after 1920. Under this group of alkaloids come such well-known drugs as atropine

and cocaine. That they contain the peculiar ring skeleton of tropane, derived by the fusion of piperidine and pyrrolidine rings, with three atoms in common (I) was definitely established by Willstätter, not only by degradation experiments, but also by careful synthesis.



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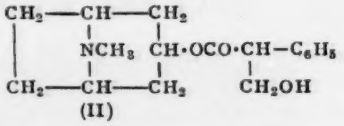

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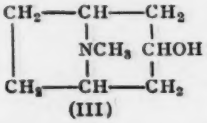

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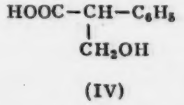
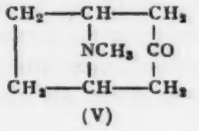


(IV)

On hydrolysis, atropine (II) gives rise to tropine (III) and tropic acid (IV). Thus the alkaloid is an ester. Of the two parts, the constitution of tropic acid was finally established by its synthesis by Ladenburg and his collaborators. Though tropine and a number of its derivatives had been studied, its constitution was not yet understood. This was definitely achieved by Willstätter by a careful examination of its oxidation products and also by laborious synthetic work. Tropine (III) is a secondary alcohol and yields the ketone tropinone (V) as a first stage in oxidation. This was first prepared by Willstätter and studied. He showed that it contained two reactive methylene groups capable of reacting with benzaldehyde, ethyl oxalate and amyl nitrite. There was thus evidence for the presence of $-CH_2CO\,CH_2-$ group in tropinone and $-CH_2-CHOH-CH_2-$ in tropine. This definitely rendered the older tropine formula of Merling untenable. Willstätter's observations could be explained on the basis of three possible formulæ, and the correct choice was made by further study of tropinic acid (VI) which is the next stage in the oxidation of tropine. He succeeded in isolating N-methylsuccinimide as a product of energetic oxidation of tropinic acid and thus definitely established the presence of a pyrrolidine ring. Further this acid contains all the carbon atoms of tropine and tropinone and when it is subjected to exhaustive methylation, it yields an unsaturated dibasic acid and subsequent reduction of it produces pimelic acid (VII). Thus it was clear that all the seven carbon atoms of tropine were embodied in a 7-atom ring. The changes were represented as below:



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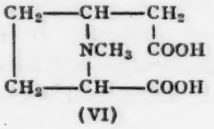

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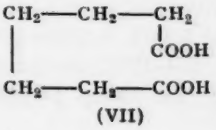
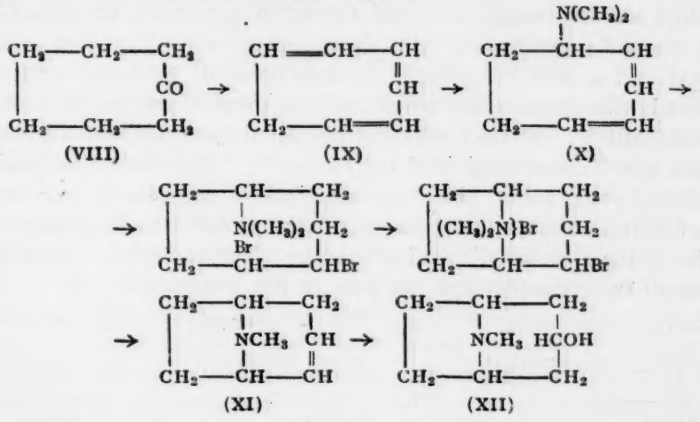
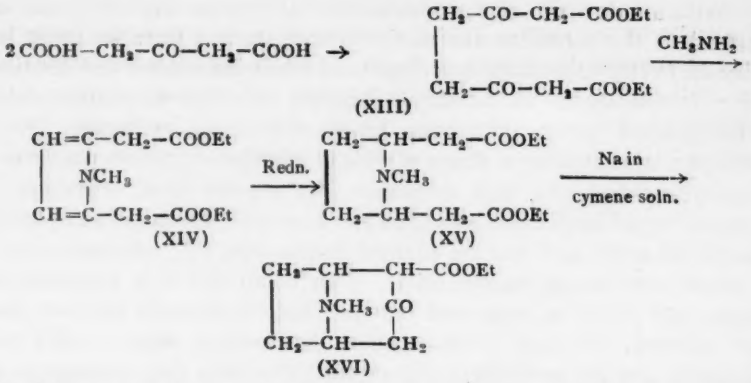


(VII)

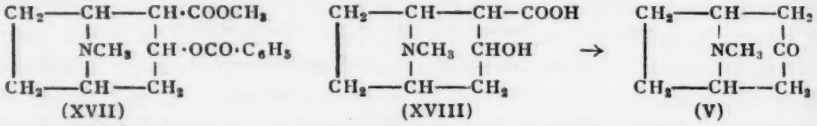
Willstätter's view of the constitution of tropine was confirmed by his synthesis of this substance and its derivatives, starting from the cyclic ketone suberone (VIII). One of the main reactions employed is what was described by him as intramolecular alkylation. It depends upon the alkylating action of a halogenated group on a basic group of the same molecule. The work involved a large number of stages of which tropilidine or cycloheptatriene (IX), α-methyltropidine (X), and tropidine (XI) are the most important. The synthetic tropidine (XI) was converted into bromodihydrotropidine by hydrogen bromide in acetic acid and the solution heated with 10% sulphuric acid when it passed into pseudo-tropine (XII). This compound is a stereo-isomer of tropine and could be converted into it. The relationship between the two basic alcohols and their interconversion had already been worked out by Willstätter and his co-workers. His original view that they correspond to *cis* and *trans* forms has received ample support.



The importance of tropinone, as a possible starting point for the synthetic production of the therapeutically valuable alkaloids, atropine, hyoscyamine, cocaine, tropa-cocaine and the artificial tropeines led Robinson (1917) to evolve a very simple method for its preparation. He found that a mixture of succindialdehyde, methylamine and acetone in water produced tropinone. A much better yield was obtained when in place of acetone, the calcium salt of acetone dicarboxylic acid was employed. This idea was taken up in Germany and a number of processes were described mostly in patent literature. Willstätter and Pfannen-stiel (1921) developed a method (shown below) by which acetone dicarboxylic acid could be converted into ethyl tropinone-carboxylate (XVI). From this tropinone, tropine and ecgonine could be easily prepared.



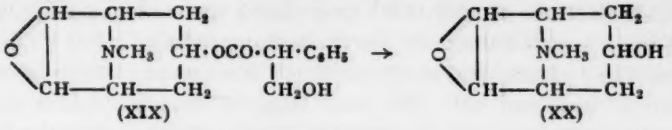
Similar to atropine, cocaine (XVII) is also an ester and yields on hydrolysis methyl alcohol, benzoic acid and a complex substance, ecgonine (XVIII), and it could be synthesised from these components. Ecgonine contains a hydroxyl and a carboxyl group. Definite proof of the relationship of this compound with tropane and tropinone was supplied by the work of Willstätter and Müller. It was found that by careful oxidation with chromic acid ecgonine can be converted into tropinone (V). This fixes the position of the hydroxyl group also. Since the behaviour of ecgonine is not that of an α- or γ-hydroxy-acid, the carboxylic group was placed in the β-position, so that ecgonine is the β-carboxylic acid of tropine. The constitution thus derived explains all the properties and reactions of the compound.



Willstätter and co-workers developed an ingenious method of introducing a carboxylic group in tropinone and this eventually led to the synthesis of naturally occurring cocaine. Tropinone forms a sodium derivative which reacts with carbon dioxide to yield sodium tropinone-carboxylate. The methyl ester of the acid, when reduced with sodium in alcohol, gives rise to a mixture containing methyl esters of *dl*-ecgonine and *dl*-pseudoecgonine. These correspond to tropine and pseudo-tropine obtained from tropinone. *dl*-Cocaine was obtained by benzoylation of *dl*-ecgonine methyl ester and resolved into its optically active components by crystallisation of the hydrogen *d*-tartrate, when the *l*-base (natural cocaine) was obtained.

Among other important contributions of Willstätter to the chemistry of this group of alkaloids may be mentioned the following: (1) The isolation

of scopine by the careful hydrolysis of hyoscine by pancreatic lipase in presence of ammonium chloride as buffer. This helped the establishment of the constitution of hyoscine (XIX) and the nature of its real (scopine XX) and apparent (oscine XXI) basic hydrolytic products. (2) The synthesis of hygric acid which led to a correct understanding of the constitution of hygrine (XXII).



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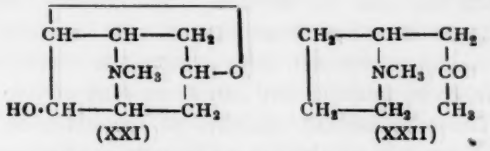
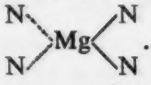


(XXI) (XXII)

Chlorophyll and Other Leaf Pigments

From 1906–1914 Willstätter and his collaborators not only succeeded in preparing pure chlorophyll for the first time, but their chemical investigations laid the foundation of our present knowledge of the green pigments. The name "Chlorophyll" was first given by Pelletier and Caventou in 1817 to the green colouring material of the chloroplast of plant leaves. Progress was however very slow. Though Stokes (1864) showed spectroscopically that the pigment was a mixture and Sorby separated it into four components—two yellow and two green—by partition between immiscible solvents, their work did not attract much attention. Due to the use of too drastic methods, many attempts that were made subsequently to study the green pigments failed. Consequently the individuality of chlorophyll, whether it consisted of one or more of related compounds, was not established. As a matter of fact one worker reported that from a single plant species alone, he had obtained twenty different chlorophylls. The analysis, qualitative as well as quantitative, was not reliable and hence the correct composition was not known.

The first serious handicap in the chemical study of chlorophyll was its ready changeability which rendered isolation of pure samples impossible. As the alternative, Willstätter had to study only the products obtained from it by the action of alkali and acid. In this line of attack he was remarkably successful and it was possible for him, before chlorophyll itself was known, to determine its characteristics from the investigation of the decomposition products so completely, that there was nothing new to be learnt by the investigation of the pure natural dyestuff, when he was able to prepare it later on.

Chlorophyll exhibits ester nature and could be easily saponified by means of alkali. The products which are called " Chlorophyllins " are acids and they contain magnesium. The linking of this metal is uncommonly stable to alkalies, but is sensitive to acids. From the chlorophyllins, carbon dioxide molecules could be split off one by one. As the result of heating with concentrated alcoholic alkali to 240°, they yield a series of crystalline, brightly coloured and intensely fluorescent decomposition products, called " Phyllins ". The names like " glauco- and rhodo-phyllins " were given to them in virtue of their colours—blue and red. The final stage of decarboxylation could be effected with soda-lime and the product " aetiophyllin " had the formula $C_{31}H_{34}N_4Mg$ and was thus free from oxygen. It was then obvious that magnesium was bound to nitrogen, since compounds in which it is linked to carbon are known to be unstable and can in no case survive treatment with boiling alkali. The extraordinary stability of the structure was attributed to the existence of secondary valencies, so that magnesium was bound to all the nitrogen atoms,  . These results indicated the important role of magnesium in plants and the necessity for adequate supply of this element in plant nutrition.

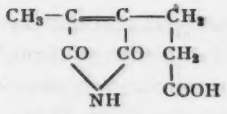
By the action of acids on chlorophyll and its derivatives mentioned above, magnesium-free compounds could readily be obtained. The phyllins gave rise to what are known as " porphyrins ". For the case of chlorophyll itself, oxalic acid was found to be the best and the compound produced by the simple removal of magnesium is known as " phæophytin ". This is a wax-like substance yielding on saponification, besides the acid part, methyl alcohol and a new unsaturated alcohol " phytol ", having the formula $C_{20}H_{40}O$. An examination of the variation in phytol content of phæophytin from various sources led to a clear understanding of what is called " crystalline chlorophyll ". This is the name of the large crystals that are developed in the chloroplasts when sections of the green leaves are soaked in alcohol. This substance contains very little phytol. Willstätter showed that it is produced by the elimination of phytol from chlorophyll and subsequent esterification of the acid product ' chlorophyllide ' with the methyl or ethyl alcohol employed and that the change is brought about by an enzyme " chlorophyllase " present in the leaves. Thus the crystalline chlorophyll is a simpler derivative of the naturally occurring compound and has a methyl or ethyl group in the place of the phytyl.

By the decomposition of phæophytin with alkali, two series of compounds phytochlorins (green) and phytorhodins (red) were obtained. These were

separated using an immiscible mixture of ether and hydrochloric acid and the "hydrochloric acid number" was employed for characterising them. Amongst these compounds, phytochlorin-e and phytorhodin-g were primary products. The former has the formula $C_{34}H_{34}O_5N_4$ and the latter $C_{34}H_{34}O_7N_4$. They are always found in a constant proportion of three to one. Two explanations of these results are possible: (1) that they are both derived from a single entity and (2) that they are obtained from two different entities present in what is called "chlorophyll". The first alternative is not possible since it will involve a molecular formula for chlorophyll four times the normally accepted value. Each molecule of phytochlorin or phytorhodin accounts for practically all the carbon atoms of chlorophyll minus phytol. There was thus conclusive evidence for the existence of chlorophyll-a and chlorophyll-b. Convenient methods were then developed for separating these chlorophylls and then extended to their derivatives. They were based on the unequal distribution of the components between different solvents, immiscible with one another.

There is no doubt about the efficiency of the preparatory methods employed by Willstätter and about the purity of chlorophyll-a and chlorophyll-b obtained by him for the first time. The chromatographic methods, developed later, are however more convenient. Chlorophyll-a has the formula $C_{55}H_{72}O_5N_4Mg$ and chlorophyll-b, $C_{55}H_{70}O_6N_4Mg$; the latter contains two hydrogen atoms less and one oxygen atom more. Detailed estimations were made of the proportions in which these two components of chlorophyll occur in plants. About 200 cases were studied and this work conclusively proved the identity of chlorophyll in all plants. There is only one leaf-green consisting of two components—chlorophylls-a and-b. The proportion is three to one; only sea-weeds form an exception to this quantitative relation. They contain mostly chlorophyll-a and only very small amounts of -b.

The carboxyl groups of the porphyrins could be removed one by one just as in the case of the phyllins. When the last carboxyl was removed by heating with soda-lime "ætioporphyrin" having the formula $C_{31}H_{36}N_4$ was obtained. This corresponds to ætiophyllin, the magnesium compound. Oxidation of phylloporphyrin yielded compounds which were already well known from the investigations of Kuster on the decomposition of hæmin. Methyl ethyl maleic imide was obtained in quantities accounting for the formation of more than one molecule and hæmatic acid (XXIII) in molecular quantity. Reduction of porphyrins gave rise to hæmopyrrol, which had been originally obtained from the blood pigment. Willstätter showed that it was a complex mixture containing phyllo (XXIV), *iso*-hæmo- (XXV) and cryptopyrrols (XXVI). It was thus clear that ætioporphyrin contained four



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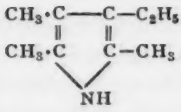

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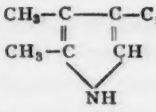

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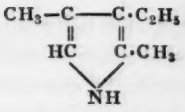
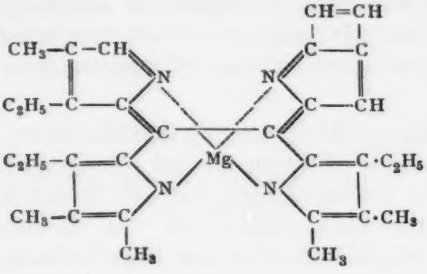
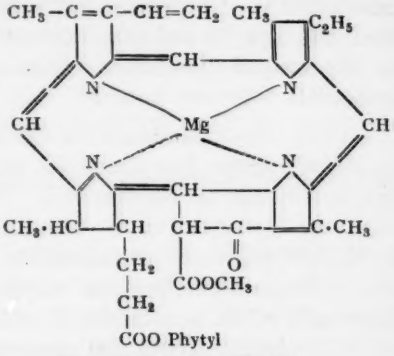


(XXVI)

pyrrolnuclei which had certain alkyl substituents. From the small number of hydrogen atoms present, the compound was expected to be highly unsaturated. Though the formulæ of ætioporphyrin and ætiophyllin suggested by Willstätter had later to be modified in many ways, it embodied the essential and characteristic features. The modern structures are based upon Kuster's suggestion of symmetrical structure for porphyrins and upon the detailed investigations of Conant and Hans Fischer.



Aetiophyllin (Willstätter)



Chlorophyll-a (Hans Fischer)

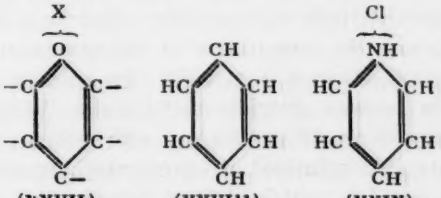
Willstätter also carried out an interesting piece of work relating to "hæmatin" derived from the blood-pigment. By the degradation of this substance also, ætioporphyrin was obtained and it was found to be identical with the sample derived from chlorophyll. This showed conclusively that the fundamental part of both pigments vitally associated with life is the same. Previous to this work, only pyrrol derivatives, more or less similar in nature, were derived from both. He realised, however, that they differed in essential details; as for example, chlorophyll is a wax and has magnesium in it, whereas hæmoglobin is a protein and contains iron. It may be necessary to record here the highly important experiments on the synthesis of chlorophyll from its components. By the action of chlorophyllase, he could get phytol to combine with chlorophyllide to yield chlorophyll, thus reversing the original hydrolytic reaction. In a different way he achieved the synthesis of chlorophyll from phæophytin by introducing magnesium through the medium of the Grignard reagent. In connection with his work on chlorophyll he also carried

out some fundamental experiments on the most important of the carotenoids—carotin, xanthophyll and fucoxanthin. Thus through what were previously dark and uncharted regions Willstätter blazed a trial with remarkable success. It has permanently dispelled darkness and made onward progress possible.

Anthocyanins

Chlorophyll and the yellow and orange carotenoids are insoluble in water and are classed as plastid pigments. The water-soluble components consist mainly of the yellow anthoxanthins and the red, violet and blue anthocyanins. Our present accurate knowledge of the last group has been gathered since 1913 in which year Willstätter and Everest published an account of their pioneering work on corn-flower blue. Though the bright colours of the flowers, leaves and fruits had attracted the attention of chemists from the time of Robert Boyle, very little progress was made and it is almost possible to neglect previous work. A formidable difficulty was the readiness with which the anthocyanins underwent change into colourless substances, a phenomenon which we now call pseudo-base formation.

The isolation, characterisation and analysis of many pure anthocyanins were effected by Willstätter and his collaborators between the years 1913–1917. The exploration and the consolidation of this new region of knowledge was remarkably rapid. Two important factors seem to have made this achievement possible; the time was ripe for these discoveries and the proper man was there to make them. As a result of the labours of A. G. Perkin and von Kostanecki, the constitution of the large number of flavones and flavonols which are built upon the C_{15} model were well understood and the products of decomposition of these substances with alkali were well known. Through the researches of Collie, Baeyer and others, the constitution of the relatively stable oxonium salts had been investigated and it was known that these invariably contain a structure known as the pyrylium (XXVII) which may be compared with benzene (XXVIII) and pyridine hydrochloride (XXIX). Willstätter came to this problem of anthocyanins with his rich experience on the study of highly unstable substances like chlorophyll. Many of the special methods used in chlorophyll chemistry appeared again in a modified



(XXVII) (XXVIII) (XXIX)

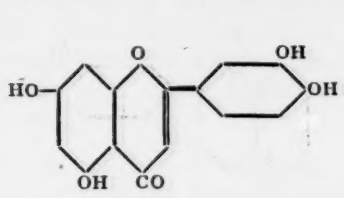
form for the examination of anthocyanins. One such is the procedure for the separation and characterisation of anthocyanidins, their mono- and di-glycosides and complex glycosides using partition between amyl alcohol and dilute hydrochloric acid.

The technique of preparing the pure anthocyanins varied from case to case. But the standard procedure depended on the recognition that they exist as salts in acid solution. The dry petals of flowers which sometimes contained about 20% of anthocyanins and thus constituted very rich sources, were extracted with methyl alcoholic or ethyl alcoholic hydrogen chloride or with acetic acid. The crude pigment salt was precipitated from the solution by the addition of a large volume of ether and this or a similar process was frequently repeated. The anthocyanin chlorides were then crystallised from aqueous or alcoholic hydrochloric acid. In a number of cases, the picrate or the lead salt was isolated as an intermediate stage in the purification. The blue corn-flowers and the red rose afforded one and the same cyanin chloride having the composition $C_{27}H_{31}O_{16}Cl$. The difference in the colours of the flowers was attributed to the influence of the pH of the sap. The scarlet pelargonium yielded a beautifully crystalline anthocyanin, having the composition $C_{27}H_{31}O_{15}Cl$. This was named pelargonin chloride.

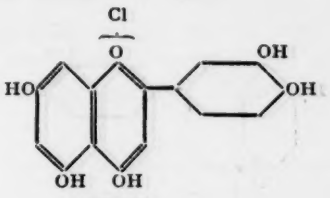
Complex as the molecules of these colouring matters undoubtedly are, the determination of their structure was made without difficulty, except for some details which were finally cleared up by later synthesis by Robinson and his co-workers. Boiling 10% hydrochloric acid brings about decomposition by hydrolysis into new coloured salts called anthocyanidins and two molecules of glucose (cyanin chloride → cyanidin chloride, $C_{15}H_{11}O_6Cl + 2\ C_6H_{12}O_6$; pelargonin chloride → pelargonidin chloride, $C_{15}H_{11}O_5Cl + 2\ C_6H_{12}O_6$).

Thus these anthocyanins are diglucosides of the anthocyanidins and the process of decomposition is one with which chemists have long been familiar. At this stage, the problem was greatly simplified; it was resolved into the recognition of the constitution of the anthocyanidins and the mode of attachment of the glucose molecules to them. Now, cyanidin chloride can easily be made to lose HCl, for example, by treatment with aqueous sodium acetate, when it yields a deep violet colour base having the formula $C_{15}H_{10}O_6$. This is also the composition of the anthoxanthin luteolin, a yellow mordant dye-stuff, derived from weld. In order to see whether there is any relationship between cyanidin and luteolin, Willstätter and Everest studied the decomposition of the former with potassium hydroxide and were able to isolate phloroglucinol and protocatechuic acid. It was already known that these could be obtained from luteolin by similar treatment.

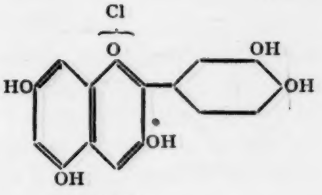
Luteolin (XXX), as a typical anthoxanthin, forms salts with strong acids. Though luteolin hydrochloride has the same composition as cyanidin chloride, it is much less stable and far less deeply coloured. Since, in harmony with the pyrylium theory, luteolin hydrochloride should have structure (XXXI), the only possible cyanidin formula is (XXXII).



(XXX)



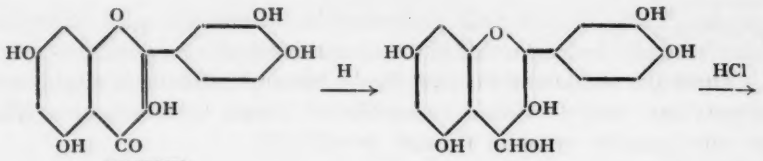
(XXXI)



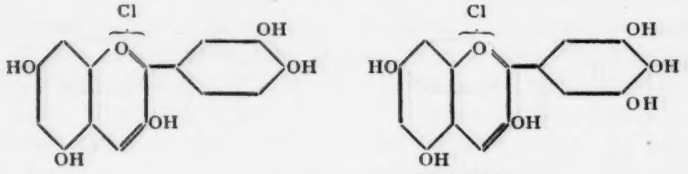
(XXXII)

This indicates that cyanidin chloride is related also to the most widely distributed anthoxanthin namely quercetin (XXXIII), and a rigid proof of the fundamental structure of the anthocyanidin was obtained when it was found that it could actually be produced by the reduction of quercetin with magnesium in acid solution. The change is represented by the formulæ given below. Further support was obtained from the synthesis of pelargonidin starting from 3: 5: 7-trimethoxy-coumarin and anisyl magnesium bromide.

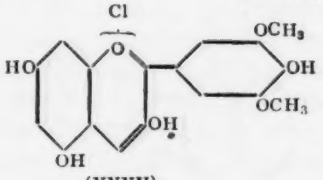
Later, Willstätter and his co-workers isolated other anthocyanins. Delphinin chloride from Delphinium flowers yields on hydrolysis delphinidin chloride, $C_{15}H_{11}O_7Cl$ (XXXIV), 2 mols. of glucose and 2 mols. of *p*-hydroxy-benzoic acid. Meco-cyanin from the red poppy is an isomeride of cyanin chloride and is also a diglucoside of cyanidin. Many mono-glucosides are known. A few are mono-galactosides and there are many rhamno-glucosides and pentose-glucosides. In complex anthocyanins as in delphinin chloride, acylation of a number of hydroxyl groups has taken place. Besides the three main anthocyanidins, others which are methyl ethers of cyanidin and delphinidin were also obtained by hydrolysis of various anthocyanins. The most widely distributed of these is malvidin which is 3′: 5′-dimethyl ether of delphinidin (XXXV).



(XXXIII)



(XXXIV)



(XXXV)

The last piece of work in this field which Willstätter undertook along with Schudel was a preliminary examination of betanin, the colouring matter of the beet. They showed that it contained nitrogen and thus it belonged to a new type of nitrogenous anthocyanins. It has been subsequently studied in greater detail by Price, Robinson and others.

The problem of the point or points of attachment of the sugar residues was not solved by Willstätter. This has been subsequently done to some extent by Karrer *et al.* but mostly by Robinson and his co-workers. The second group of investigators have developed excellent methods for synthesising not only the anthocyanidins, but the anthocyanins themselves and have further elaborated the chemistry of the anthocyanins in various directions, such as the survey of anthocyanins and the genetics of flower colour. But it is certainly correct to say of Willstätter that by his pioneering researches on the anthocyanins, he opened, and all but closed, a new chapter of organic Chemistry.

Enzymes

The later years of his scientific career were spent on the study of enzymes. The most labile and the most elusive of chemical entities were then best tackled, his vast previous experience with unstable and complex substances being put in with great effect. Some very remarkable and revolutionary results

were obtained by him and he has left behind not only his discoveries, but also an important school of workers.

Generally enzyme research has been concerned with the study of soluble enzymes or soluble part of enzymes. There are, however, enzymes or portions of enzymes which cannot be attacked by simple processes of solution and which can be demonstrated to be present in organ, tissue or cell residues. These enzymes which are chemically bound to the protoplasm and which are insoluble because of the chemical composition of these complexes, were named desmoenzymes as distinguished from soluble or lyoenzymes.

As he has himself expressed in one of his lectures, he unwittingly entered the realm of desmoenzymes when comparing the chlorophyll of a variety of plants. Chlorophyll from more than two hundred plants of different classes and geographic origin were examined and were eventually shown to be identical. But in earlier investigations, great variations in the phytol percentages had been found. Instead of 33% only 17, 6, 2 and even 1% were obtained in many cases. The plants with chlorophyll of apparently low phytol content gave rise to what is known as crystallised chlorophyll. The puzzle was solved by a careful study of the process of extraction. When chlorophyll is rapidly withdrawn from the leaves, and the solution at once separated from the leaf residue, then the phytol content is always normal. But if the alcoholic solution of the pigment remains in prolonged contact with the leaves, the phytol percentage drops more or less, sometimes almost to zero. In such cases crystallised chlorophyll results, due to replacement of the phytyl group by ethyl. This reaction is brought about by the enzyme chlorophyllase which accompanies chlorophyll in the leaves. In aqueous medium it causes hydrolysis to free carboxylic acids, the chlorophyllides. Even the partial synthesis of chlorophyll from these acids and phytol was accomplished by means of this enzyme. Chlorophyllase is a typical desmoenzyme and it remains in the leaf residue after treatment with all solvents. The leaf residues have definite enzymatic character. They react only with ester chlorophyll, become inactive after thorough washing, and addition of calcium restores activity (distinctive characteristic).

The above work led on to a detailed investigation of castor bean lipase as an example of desmoenzyme. Effect of reagents or other enzymes caused marked changes in the nature of this enzyme and the characteristic transformations were interpreted as partial proteolysis of the colloid carrier. From these evidences as also from the results of specific adsorption methods for the concentration and separation of lyoenzymes he developed his conception of

the structure of enzymes, as consisting of a specific active group with a high molecular colloidal carrier.

A careful study of the saccharase of yeast revealed that it belonged to a different type of enzymes which were called " endoenzymes ". Saccharase was first noted to become soluble through a definite enzymatic process and was therefore considered to be tied to the protoplasm. But later it was realised that purely mechanical destruction of the cell membrane was sufficient to enable the enzyme to pass into solution. Hence there was proof that it was simply protected or tucked away by the cell membrane. Enzymes of this type, protected from solution by mechanical and not by chemical means, were classed as " endo-enzymes ".

A detailed study of the extraction of pepsin and trypsin indicated their existence in lyo- and desmo-forms. Deeper insight into the complexity of the desmoenzymes, which could be extracted in fractions, was obtained by the study of leucocyte amylase. He came to the conclusion that chemical changes taking place during autolytic processes concern not only the substrates, but the enzymes themselves and influence their essential characteristics. All these results found satisfactory explanation in his theory of enzyme constitution as made up of active groups and colloidal carriers which are capable of being changed. There are a few cases where specific active groups postulated by the theory have been chemically defined. Respiratory ferments have been recognised by Warburg and Keilin as iron porphyrin complexes with unknown colloidal carriers. Similarly catalase and peroxidase are claimed to owe their activity to the presence of similar groups. More recently coenzymes based on the vitamins of the B group are considered by some to be the prosthetic groups of enzymes. Other workers, however, seem to feel that this is an assumption for which there is insufficient evidence and that other explanations are possible.

In Willstätter's opinion the crystalline enyzmes of American workers were not pure enzymes which were expected by him to be far more reactive. In calculating their efficiency, he and Haber were led on to the application of chain reaction mechanism to enzyme actions and this has come into general use.

A number of other items of work were carried out by Willstätter in the course of his long and eventful career. As the more important of these may be mentioned the study of benzene constitution, quinonoid compounds, catalytic hydrogenation and carbon dioxide assimilation. Among his numerous writings, those that have been published in book form are (1) Untersuchungen über chlorophyll, (2) Untersuchungen über die Assimilation der Kohlensaure and (3) Über die Enzyme Zeugnisse.

Willstätter has been one of the most famous and highly respected personalities during the past fifty years and his name will live for ever as a brilliant star in the firmament of chemical history. He was awarded the Nobel Prize in 1915 for his work on leaf and flower colouring matter. Academies and chemical societies all over the world vied with each other in honouring him and he was one of the earliest of the honorary members of the Indian Academy of Sciences. However, due to the development of Nazi tyranny and mass hysteria in Germany, his life became difficult in his own country and he narrowly escaped being sent into a concentration camp. The last years of his life were mostly spent outside Germany and he died in Locarno-Muralto in Switzerland, a few days before his 70th birthday.

T. R. SESHADRI.

PULSATIONS ON MERCURY SURFACE CAUSED BY POLARISATION

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Received April 30, 1943

If an iron wire is brought into contact with the surface of mercury, kept under a dilute solution of sulphuric acid, containing a little potassium dichromate, the mercury begins to pulsate.[1] This interesting phenomenon has been referred to as the " Mercury Heart ". Its exact mechanism has been a puzzle, as can be gathered from the following observation of Thompson[2]: " Why iron and chromate are the only means of producing this effect, however, does not seem to have been explained."

The investigations described in this paper have elucidated the phenomenon. Mercury in contact with a solution containing acid and an oxidising agent, becomes anodically polarised. If the solution contains chloride ions, the polarisation becomes visually evident by the formation of a film of calomel. Such a surface has comparatively a low interfacial tension owing to electro-capillary effect. If an iron wire is brought into contact with the surface, a voltaic cell is formed, with iron as the anode and mercury as the cathode. The mercury now tends to get cathodically polarised and the interfacial tension rises. The mercury drop consequently contracts laterally. If the iron wire is touching at a side of the drop, the drop detaches itself from the iron wire, in the process of contraction. The mercury, now no longer in contact with iron, is once again anodically polarised and the interfacial tension, once again falls, leading to a lateral dilatation of the drop and re-establishment of the contact with the iron wire. The process gets repeated and thus leads to pulsations in the mercury.

In a study of the phenomenon, it was observed that dichromate can be replaced by other oxidising agents such as hydrogen peroxide or potassium permanganate. With hydrogen peroxide, addition of a trace of ferric chloride, greatly quickens the pulsations. It is well known that the oxidising action of hydrogen peroxide is promoted by iron salts.

That the modification of the surface by contact with the iron wire is due to cathodic polarisation has been established from the following observations: (*a*) The mercurous chloride film, which as already stated, is formed

on the surface of mercury in presence of hydrochloric acid and an oxidising agent, disappears when the iron wire is placed at the interface between mercury and solution. The disappearance of the film can be observed visually. It takes place very quickly over the entire surface and can only be due to cathodic reduction. (*b*) The pulsations can also be obtained in the following way:—An iron wire is dipped into the solution and connected externally to a graphite rod (the " lead " of a pencil) and the latter is brought into contact with the mercury/solution interface. (*c*) It is possible to obtain pulsations, without the use of iron or other base metal, by employing two graphite rods, dipping in the solution and connected to a potentiometric arrangement, the cathodic rod being in contact with the mercury.

These results indicate that a similar phenomenon should be observed with a reducing agent in place of the oxidising agent, the graphite rod touching the interface however, being *anodically* polarised. We have been able to produce the expected effect, using a reducing agent such as stannous chloride or titanous chloride. It is of interest to note that if an " arc carbon " is used instead of graphite, in this experiment, the pulsations persist for some time, even after the polarising E.M.F. has been cut off. This is probably due to chlorine occluded by the carbon rod, which makes it function as a chlorine electrode for a time, after the E.M.F. is removed. Another interesting observation was made. Carbon rods, on being worked in the arc for some time, were found to create pulsations in mercury (under reducing solutions) even without any anodic polarisation. The effect, though rather feeble, persisted for considerable time. It is probable that at the temperature of the arc, an oxidising agent is produced in the rod. We noticed that a carbon rod, after working in an arc lamp, produces a thick film of calomel, when brought at the interface: mercury/dilute hydrochloric acid.

It is of importance to note that to secure pulsations in mercury under an oxidising agent, the iron wire (or the cathodically polarised electrode) must contact the mercury at the *side* of the drop. Under reducing agents however, the mercury has to be touched at the *apex* of the drop, by the anodically polarised electrode. This difference in procedure is rendered necessary by the fact that in our experiments, when cathodically polarised electrodes contact mercury kept under an oxidising medium, the interfacial tension *increases* bringing about an immediate lateral *contraction*. On the other hand, the interfacial tension *decreases* and a lateral *expansion* is produced, when an anodically polarised electrode touches mercury kept under the reducing solution.

Finally it may be noted that pulsations in mercury can be brought about even without the use of any oxidising or reducing solution. The following procedure

is adopted. The mercury is kept under an aqueous solution of an electrolyte (*e.g.* dilute sulphuric acid). Two graphite electrodes (" Lead " pencils) connected to a source of E.M.F. are used. The anode is kept vertically above the drop and the cathode kept at the side. The distances are so arranged that both the electrodes do not come into contact with the mercury drop *at the same time*. A resistance is used in series with the electrodes so as to avoid any accidental short-circuiting. The pulsations are effectively produced by employing about one c.c. of mercury in a shallow basin with a slight curvature at the bottom, and covering it with 0·2 N sulphuric acid. A potential of one to two volts is applied. It is found that the pulsations persist even when a resistance of over 10,000 ohms is included in the circuit.

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2. Thompson .. *Theoretical and Applied Chemistry*, 1939, 107.

OPACITY CHANGES IN GEL-FORMING MIXTURES DURING SETTING

Part I. Thorium Molybdate, Stannic Arsenate and Silicic Acid Gels

By Mata Prasad and V. S. Gogate

(From the Chemical Laboratories, Royal Institute of Science, Bombay)

Received April 12, 1943

The transparency or opacity measurements of colloidal solutions were first made by Mukherjee and co-workers during their study of the kinetics of the coagulation of sols by electrolytes. In their earlier studies they measured[1] the intensity of light transmitted through the coagulating system in various spectral regions by means of a spectrophotometer. Later on, Mukherjee and Majumdar[2] measured the transparency of the coagulating sol by allowing the transmitted light to fall on a thermopile connected to a sensitive galvanometer. The use of this method was extended by Prasad and Hattiangadi[3] for the study of the kinetics of the setting of silicic acid gels.

Desai[4] modified Mukherjee and Majumdar's method by substituting a photo-cell in place of thermopile. According to him a photo-cell is decidedly more sensitive and reliable than the thermopile as it is easier to eliminate stray light than stray heat. Desai's photo-cell method was modified by Prasad and Modak[5] who magnified the current generated in the photo-cell by a single stage amplification unit.

The photo-cell methods of Desai and co-workers and Prasad and Modak require that the current feeding the source of light should be kept constant throughout the investigation. These co-workers used the current from the main supply and corrected it for any fluctuation by means of a potentiometric arrangement. A second necessary requirement of their apparatus arising out of the photo-cell arrangement is that the potential applied to excite the cell should be kept constant throughout. This was achieved by using storage cells giving constant voltage. Any change in the constancy of these two factors will introduce large errors and would lead to wrong conclusions.

An apparatus described in the following pages was assembled with a view to get over all the possible errors mentioned above by an auto-controlled device and it was employed to measure the changes in opacity taking place during the setting of mixtures giving rise to gels of thorium molybdate, stannic arsenate and silicic acid.

Experimental

Description of the apparatus—

The apparatus mainly consists of three parts:

(*a*) Rectifying circuit.

(*b*) Arrangements for maintaining the source of light and photo-cells under constant conditions.

(*c*) Arrangement for measuring the current.

(*a*) *Rectifying circuit.*—This is the usual rectifying arrangement which converts 230 A.C. into D.C. at about 400 volts and 180 milliamps. It was provided with the filter system to smoothen the current output. The D.C. current thus obtained was used in the arrangements described under the heading (*b*).

(*b*) *Source of light.*—The circuit diagram of this part of the apparatus is shown in Fig. 1. In this diagram

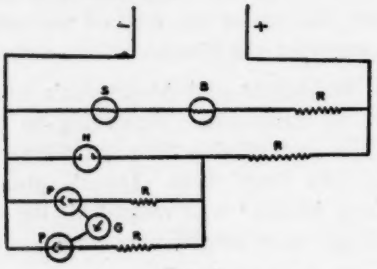

FIG. 1

S = Source of light (lamp).
B = Regulating valve (Barreter's filament lamp).
N = Neon stabilizer.
P = Photo-cell.
G = Galvanometer.
R = High resistances.

The source of light is supplied with constant amperage by means of the regulating valve B which is put in series with it and which controls automatically the small fluctuations occurring in the mains. The voltage applied to the photo-cells is autocontrolled by the neon-stabiliser N which is put in parallel with the photo-cells as shown in the figure. It was found that a change in the main A.C. current by about 10% causes no appreciable changes in (1) the current feeding the source of light and (2) the voltage applied to the photo-cells.

In order to make this arrangement direct reading, the source of light S was placed between the two photo-cells (P) of the same type. They were connected in parallel as shown in Fig. 2. The neon-stabiliser, the source

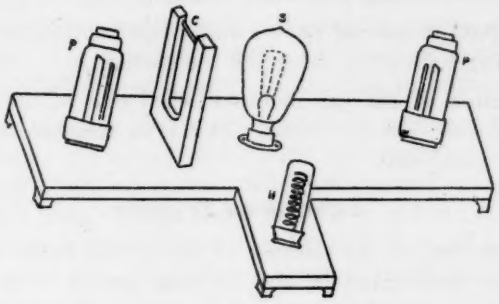

FIG. 2

of light and the photo-cells were mounted on a suitable board and their positions were fixed in order to avoid any discrepancies creeping in the results on account of the shifts in their respective positions. C is a glass cell mounted on a wooden base, employed to contain the gel-forming system. The glass cell exactly fits in the base and can be removed easily from it for cleaning purposes. The regulating valve B becomes very hot when the current is put on and it was found to interfere with the results when mounted on the same board. Consequently it was mounted on a separate base and was kept in a distant corner.

The distances between the source of light and the photo-cells were so adjusted that when the glass cell is empty there is practically no initial current flowing through the galvanometer. Any changes noted by the galvanometer after introducing a gel-forming mixture in the glass cell are therefore exclusively due to the changes taking place in the system with time.

(*c*) *The measuring instrument.*—This part of the apparatus consists of a sensitive moving coil galvanometer of deat-beat type. The deflections of the galvanometer were measured on a scale placed at a suitable distance from it.

The apparatus described above was kept in an air thermostat maintained at a constant temperature, since the rate of gelation has been found to alter with a change in temperature. The galvanometer took nearly one hour to reach a steady state, after switching on the current; hence all observations were taken after this interval.

The source of light is constantly kept on during the period of observations and thus photo-cells always remain in a steady state.

The current produced by the photo-cell is very small and hence extreme care was taken to avoid any possible leakage.

All the apparatus was set up in a dark room and extreme care was taken to keep all stray light away from the thermostat.

The deflection of the galvanometer when one of the photo-cells was completely covered was 11·1 cms. This is a measure of the total light falling on the photo-cell.

Experimental Procedure

Varying amounts of the solution of one of the constituents of the gel-forming mixture were taken in one test-tube and in other test-tube were taken varying amounts of the other constituents and the volumes in each test-tube were made up to 4 c.c. by the addition of the required amount of distilled water. The test-tubes were properly corked and kept in the thermostat for half an hour. The contents of one test-tube were then poured into the other and back again and this was repeated five times. A stopwatch was started just at the time of mixing. The gel-forming mixture was then poured in the glass cell which was covered with a glass slide and placed in its socket. The glass door and the black curtain of the thermostat were then put on and the position of the spot of light on the scale was noted. All this process took one minute since the commencement of the mixing of the constituents of the gel-forming mixture. The reading taken after the first minute has been taken as the zero reading. No error is involved in this process as it has been observed that during the first few minutes the changes in opacity are extremely small. The deflections of the galvanometer were then noted at various intervals of time till they reached a constant value.

White light was employed throughout. The constancy of the initial reading of the galvanometer with the empty cell and the repeatability of the results were tested from time to time.

After each set of readings the glass cell was thoroughly washed and completely dried. It was then kept in the thermostat for about 20 minutes so that it may attain the temperature of the thermostat.

1. Thorium Molybdate Gels

These gels were prepared by the method of Prasad and Desai.[6] The following solutions were used:

(A) Solution of thorium nitrate containing 48·14 g. per litre.
(B) Solution of potassium molybdate containing 100 g. per litre.
(C) 0·2 N HCl.

The effects of the addition of different amounts of (A), (B), (C) and of non-electrolytes on the opacity changes during the course of gel-formation were investigated. The results are shown by means of curves obtained by plotting observed deflections in cms. against time in minutes.

The effect of thorium nitrate was studied by adding 1·5 to 3·5 c.c. of (A) to the solution containing 0·6 c.c. of (B) and 0·5 c.c. of (C). The effect of potassium molybdate was studied by adding 0·4 to 1·0 c.c. of (B) to 0·5 c.c. of (C) before it was added to 4·0 c.c. of (A). The effect of HCl was studied by adding 0 to 0·25 c.c. of (C) to 0·6 c.c. of (B) before it was added to 4·0 c.c. of (A). The effect of methyl, ethyl and propyl alcohols were studied by adding 0 to 2 c.c., 0 to 1·5 c.c., 0 to 2·0 c.c. of the respective alcohols to a mixture of 0·6 c.c. of (B) and 0·5 c.c. of (C) before it was added to 4 c.c. of (A).

Some of the results obtained are shown in Figs. 3 and 4.

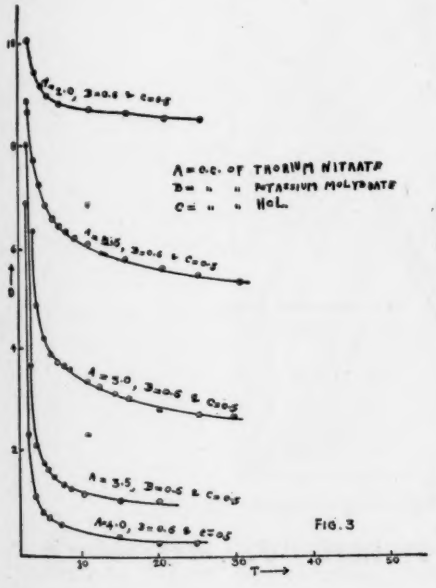

FIG. 3

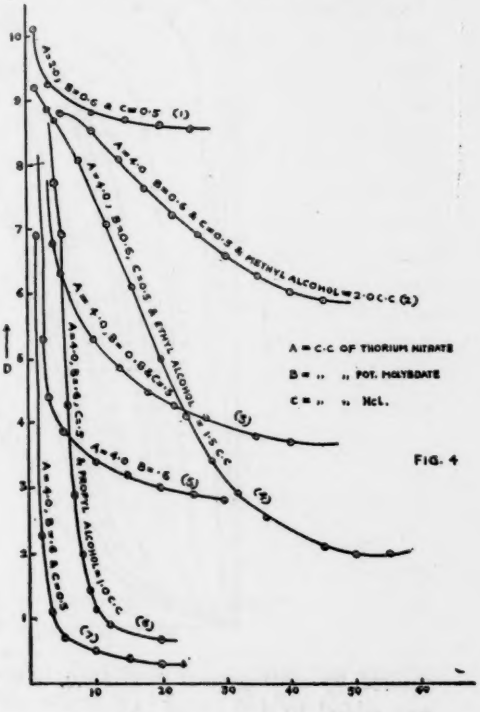

FIG. 4

2. Stannic Arsenate Gels

These gels were prepared by the method of Prasad and Desai (*loc. cit.*) by mixing different amounts of the following solutions:—

(A) Stannic chloride solution containing 195·28 g. of the salt (Merck) and 4·0 c. c. of nitric acid per litre. (Nitric acid is added to prevent hydrolysis.)

(B) 10% of solution of pyro-arsenic acid (Merck).

The effect of stannic chloride was studied by adding 2·5 to 3·75 c.c. of (A) to 0·3 c.c. of (B). The effect of pyro-arsenic acid was studied by adding 0·2 to 1 c.c. of (B) to 4·0 c.c. of (A). The effects of methyl, ethyl, propyl alcohols, glycerine and pyridine were studied by adding 0 to 1·0 c.c. of the various alcohols and glycerine and 0 to 0·2 c.c. of pyridine to 1·0 c.c. of (B) before it was added to 4·0 c.c. of (A). The effect of HCl was

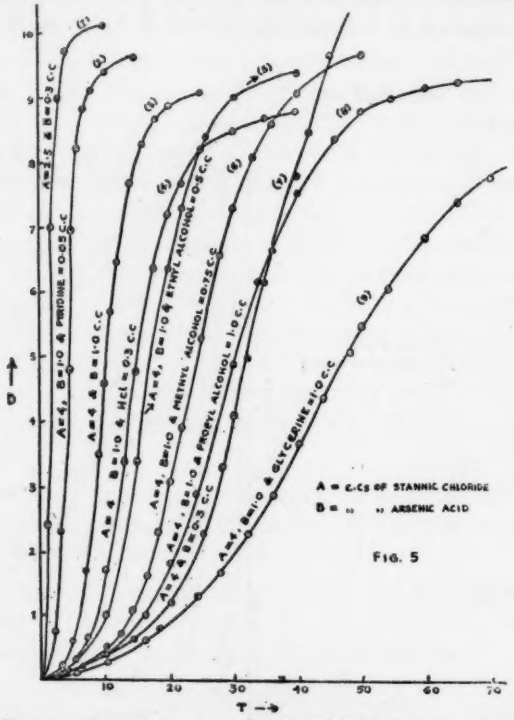

FIG. 5

studied by adding to 0 to 0·5 c.c. of 0·2 N HCl to 1·0 c.c. of (B) before it was added to 4·0 c.c. of (A).

Some of the results obtained are shown in Fig. 5.

3. Silicic Acid

These gels were prepared by mixing solutions of sodium silicate (A) (25%) and 20% ammonium acetate (B).

The effect of sodium silicate was studied by adding 2·2 to 3·0 c.c. of (A) to 4·0 c.c. of (B). The effect of ammonium acetate was studied by adding 3·0 to 4·0 c.c. of (B) to 2·2 c.c. of (A). The effect of HCl was studied by adding 0 to 0·2 c.c. of 0·2 N HCl to 2·2 c.c. of sodium silicate before it was added to 3·25 c.c. of ammonium acetate. The effect of methyl, ethyl, propyl alcohols, glycerine were studied by adding 0 to 0·4 c.c., 0 to 0·3 c.c., 0 to 0·4 c.c. and 0 to 0·2 c.c., respectively, of the alcohols and glycerine to 2·2 c.c. of (A) before it was added to 3·0 c.c. of

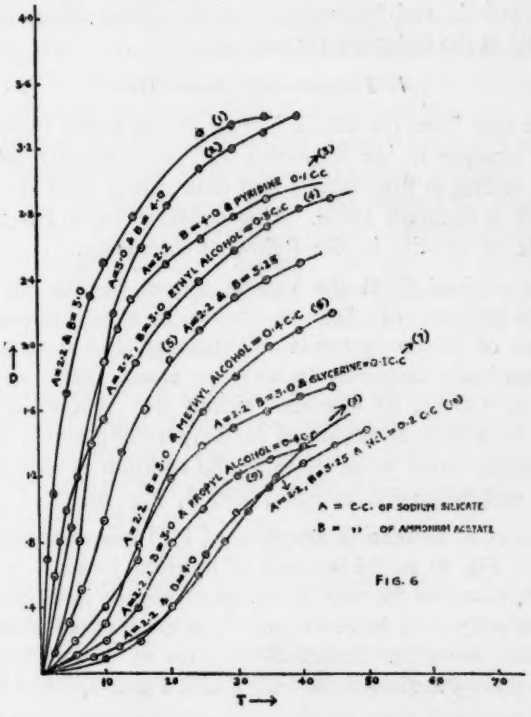

FIG. 6

(B). The effect of pyridine was studied by adding 0 to 0·15 c.c. of the same to 2·2 c.c. of (A) before it was added to 4·0 c.c. of (B).

Some of the results indicating the various effects are shown in Fig. 6.

Discussion of Results

It was observed that all the results given in the foregoing pages are fairly reproducible; several observations taken with the same gel-forming mixture under identical conditions did not differ by more than ± 2%. These results are therefore important as they represent fairly accurately the changes in opacity taking place in the gel-forming mixtures during setting.

It will be seen that all the opacity–time curves shown in various figures are smooth and continuous. This shows that the process of gel-formation, as revealed by opacity changes, is a continuous one.

The several curves in various figures show that the opacity values of a gel-forming mixture at different intervals of time change with a change in the amounts of the constituents of the gel-forming system and by the addition of alcohols and hydrochloric acid. These effects have been discussed severally in the following for each gel.

1. Thorium Molybdate Gels

It will be seen from the curves in Figs. 3 and 4 that thorium molybdate gels are very opaque in the beginning and their opacity decreases during the course of setting at first rapidly and then slowly until after sometime it tends to reach a constant value. This constant value has been called as the final value of opacity in the following discussion.

It will be noticed from the various curves in Fig. 3, that with an increase in the amount of thorium nitrate in a gel-forming mixture the rate of change of opacity systematically increases and the final value of the opacity systematically decreases to such an extent that the gel formed in the presence of 4·0 c.c. of thorium nitrate are practically water clear. The observed behaviour is attributed to the peptising action of the thorium ions. The reverse effect takes place by the addition of increasing amounts of potassium molybdate (*cf.* curves 7 and 3, Fig. 4).

The addition of increasing amounts of HCl causes the same effects (*cf.* curves 7 and 5, Fig. 4) as the increase of thorium ions (*cf.* curves 7 and 1, Fig. 4), that is, increases the rate of change of opacity and decreases the final value of the opacity to a large extent. The addition of methyl, ethyl and propyl alcohols, however, decreases the rate of change of opacity and makes the gels more opaque (*cf.* curves 7 and 2, 7 and 4, 7 and 6, respectively, Fig. 4).

2. Stannic Arsenate Gels

It will be noticed from the curves in Fig. 5, that stannic arsenate gels, unlike thorium molybdate gels, are transparent in the beginning and become

opaque during setting. The opacity increases at first rapidly and then slowly until it tends to reach a constant value, the opacity–time curves being S-shaped. Further it will be noticed that the rate of change of opacity decreases on increasing the amounts of stannic chloride (*cf.* curves 1 and 7), decreasing the amounts of arsenic acid (*cf.* curves 3 and 7) and by the addition of HCl (*cf.* curves 3 and 4) and methyl (*cf.* curves 3 and 6), ethyl (*cf.* curves 3 and 5), and propyl (*cf.* curves 3 and 8), alcohols and glycerine (*cf.* curves 3 and 9). However, the addition of increasing amounts of pyridine increases the rate of change of opacity (*cf.* curves 3 and 2).

3. Silicic Acid Gels

It will be seen from the curves in Fig. 6 that like stannic arsenate gels, the gels of silicic acid are transparent in the beginning and become opaque during setting. Some of the opacity–time curves first rise rapidly and then slow down and tend to reach a constant value while others are S-shaped. These results are in conformity with those obtained by Prasad and Hattiangadi.[7] The different curves in Fig. 6 show that the rate of change of opacity is decreased and the gel becomes more transparent as the amount of ammonium acetate in the gel-forming mixture is increased (*cf.* curves 1, 5 and 8) and HCl (*cf.* curves 5 and 10) and methyl (*cf.* curves 1 and 6), ethyl (*cf.* curves 1 and 4) and propyl (*cf.* curves 1 and 9) alcohols and glycerine (*cf.* curves 1 and 7) are added. However, the rate of change of opacity increases and the gel becomes more and more opaque as the amounts of sodium silicate (*cf.* curves 2 and 8) and pyridine (*cf.* curves 3 and 8) in the gel-forming mixture are increased.

The observed change in opacity may be attributed to the changes in the intensity of light scattered and absorbed by the micelles in the gel-forming mixtures during setting. The intensity of light scattered by colloidal particles is given by the mathematical expression developed by Lord Rayleigh

$$I = \frac{9\,N\,\pi^2\,V^2\,A^2}{\lambda^4\,X^2}\left(\frac{n_1^2 - n_2^2}{n_1^2 + 2\,n_2^2}\right)\sin^2\theta,$$

in which the terms involved have the usual meaning.

This equation reduces to $I = KNV^2$ where N and V are the number and volume of the particles. So the changes in opacity that take place during gelation are due to the changes in the values of N and V.

The changes in the intensity of scattered light may also be due to the changes in the anisotropy, distribution, and arrangement of the micelles in the gel. D. S. Subba Ramiah's measurement of depolarisation factors (ρ_u, ρ_v and ρ_h) have shown[8] that the particles in gold sols are anisotropic.

K. Subba Ramiah has shown that changes in the anisotropy of the micelles of silicic acid gels take place during setting.[9] It is probable that changes in the anisotropy, distribution and arrangement of the micelles in the gels studied in this investigation may be taking place during setting and on changing the constituents of the gel-forming mixtures and these changes may also contribute to the rate of change and final values of opacity observed, in addition to changes in the values of N and V during gel-formation, mentioned above.

The authors are grateful to Dr. B. B. Deshpande, Head of the Physics Department, Ramnarain Ruia College, Bombay, for his help and suggestions in the construction of the apparatus.

Summary

The opacity changes during the setting of thorium molybdate, stannic arsenate and silicic acid gels have been investigated by an improved apparatus assembled by the authors. The effects of the addition of different amounts of the gel-forming constituents, HCl and non-electrolytes on the gels, have been investigated and the results discussed. It has been suggested that changes in the number, size, distribution, arrangement, etc., of the micelles of the gel contribute to the observed changes in opacity.

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488-43 Printed at The Bangalore Press, Mysore Road, Bangalore City, by G. Srinivasa Rao, Superintendent, and Published by The Indian Academy of Sciences, Bangalore.